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BAC-derived SSR markers chromosome locations in cotton

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Abstract Bacterial artificial chromosome (BAC) libraries with large DNA fragment inserts have rapidly become the preferred choice for physical mapping. BAC-derived microsatellite or simple sequence repeats (SSRs) markers facilitate the integration of physical maps with genetic maps. The objective of this research was to identify chromosome locations of the BAC-derived SSR markers in tetraploid cotton. A total of 192 SSR primer pairs were derived from BAC clones of an Upland cotton genetic standard line TM-1 (*Gossypium hirsutum* L.). Metaphor agarose gel electrophoresis results revealed 76 and 59 polymorphic markers between TM-1 and 3–79 (*G. barbadense*) or *G. tomentosum*, respectively. Using deletion analysis method, we assigned 39 markers out of the 192 primer pairs to 17 different chromosomes or chromosome arms. Among them, 19 and 17 markers were localized to A-subgenomes (chromosome 1–13) and D-subgenomes (chromosome 14–26), respectively. The subgenome status for the remaining three markers remained unclear due to their two potential chromosome locations achieved by tertiary monosomic stocks deletion analysis. Chromosomal assignment of these BAC-derived SSR markers will help in integrating physical and cotton genetic linkage maps and thus facilitate positional candidate gene cloning, comparative genome analysis, and the coordination of chromosome-based genome sequencing project in cotton.

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Abbreviations

BAC	Bacterial artificial chromosome
EST	Expressed sequence tag
FISH	Fluorescence in situ hybridization

QTL	Quantitative trait locus
SCAR	Sequence characterized amplified region
SSR	Simple sequence repeat
SNP	Single nucleotide polymorphisms
TMB	TM-1 BAC/BIBAC
NTN	Tertiary monosomic cytogenetic stock

Introduction

Microsatellites or simple sequence repeats (SSRs) are considered desirable markers in molecular mapping because of their distribution throughout the genomes, hypervariability, abundance, reproducibility, Mendelian inheritance, and most importantly their amenability for high-throughput PCR-based assay for genotyping. A large number (more than 5,000) of microsatellites had been developed and are described in cotton (*Gossypium* spp.) microsatellite database (CMD, http://www.cottonmarker.org/) (Blenda et al. 2006). To date, several genetic linkage maps consisting mainly of SSR markers have been constructed in cotton (Han et al. 2004, 2006; Nguyen et al. 2004; Lacape et al. 2005; Park et al. 2005; Frelichowski et al. 2006; Guo et al. 2007). However, there are still great needs for SSR markers in the construction of a high density molecular map for reliable detection, mapping, and estimating gene effects of important agronomic traits. A high resolution genetic map is also important for genome sequence assembling and genome structure and evolution revelation.

Libraries of large DNA inserts are an essential tool for genome research including physical mapping, map-based cloning, gene structure and function analysis. Bacterial artificial chromosome (BAC) libraries with large DNA inserts have rapidly become the preferred choice for physical mapping (Hanson et al. 1995; Zhang et al. 2004). In cotton, BAC libraries have been constructed and used for physical mapping and chromosomal localization of linkage group (Frelichowski et al. 2006; Wang et al. 2006; Yin et al. 2006). Generating SSR markers from a BAC library (BAC-derived SSR markers) could provide additional advantages over other no-targeted methods for marker development (Yu et al. 2002a, b). Firstly, it has the potential to integrate physical maps with genetic maps (Danesh et al. 1998; Cregan et al. 1999; Chen et al. 2002; Yu et al. 2002a, b; Wu et al. 2004). Secondly, it is easy to convert to other types makers such as SCAR (Guo et al. 2003) and SNP (Rong et al. 2004; An et al. 2007) based on the adjacent sequences of the large inserts. Thirdly, it can streamline high-resolution mapping and map-based cloning of genes and QTLs (quantitative trait loci) of interest. Lastly, chromosome locations of BAC-derived SSR markers can provide a foundation for chromosomal assignment of BAC clones and thus facilitate chromosome-based sequencing.

Yu et al. (2002a, b) reported the discovery of a set of BAC-derived SSR markers from a TM-1 library, which were then used for molecular mapping in cotton. In this study, we conducted comprehensive cytogenetic stocks-based deletion analysis to assign these makers to specific chromosome or chromosome arm. The results provide information of chromosome locations of these markers and served as verification to the linkage mapping results. In addition to saturating current cotton linkage maps, this set of BAC-derived SSR markers' chromosomal location will facilitate map-based cloning, comparative genome analysis, and the coordination of chromosome-based genome sequencing projects.

Materials and methods

Plant materials and DNAs isolation

Three different tetraploid species *G. hirsutum* L. (AD_1) Texas Marker-1 (TM-1, CMD01), *G. barbadense* L. (AD_2) (3–79, CMD02), and *G. tomentosum* Nuttall ex Seemann (AD_3) (CMD11), an introgression breeding source, were used as plant materials for polymorphism screening. Two sets of primary monosomic and monotelodisomic F_1 interspecific hybrids, from *G. hirsutum* (TM-1) aneuploids crossed with 3–79 (*G. barbadense*) versus *G. tomentosum* and one set of tertiary monosomic hybrids (represented by NTN), from crosses with *G. tomentosum* only were used to localize the BAC-derived SSR markers to chromosome. The primary monosomic plants ($2n = 51$) lacked an entire chromosome of TM-1 complement, whereas monotelodisomic plants ($2n = 52$) lacked most or all of just one TM-1 chromosome arm. Each tertiary monosomic plant lacked a chromosome segment of two chromosomes involved in reciprocal translocation. The specific aneuploid F_1 plants and their TAES (Texas Agricultural Experiment Station) plant identification codes are listed in Table 1. All plant materials were grown

Table 1 Hypoaneuploid F_1 interspecific chromosome substitution stock identification codes

Chromosome	*G. barbadense*[a]			*G. tomentosum*[b]		
	M[c]	sh[d]	Lo[e]	M[c]	sh[d]	Lo[e]
1	–	–	9708030.04	200108072.06	200108076.10	200100323.12
2	–	–	9708030.06	200108072.08	200108077.03	200100324.03
3	–	9508049.06	9800215.05	–	200108077.09	200108077.05
4	000811.09	9508049.08	9200226.02	20018073.05	200100325.02	200108078.02
5	–	–	9708031.04	–	–	200108078.06
6	9808016.09	9508050.01	9708031.05	200108078.07	200108078.09	200108078.08
7	0008046.04	9908046.10	0.11453.15	200108073.09	200108079.02	–
8	–	–	0100463.17	–	–	200100443.02
9	9808017.07	–	9808021.10	200108074.08	–	200108079.04
10	0008046.07	0000851.09	9708032.02	200108074.10	–	–
11	–	–	9908047.09	–	–	200108080.04
12	9200331.08	–	–	–	–	200108080.07
13	–	–	–	–	–	–
14	–	–	9708032.06	–	–	200108080.10
15	–	–	–	–	–	200108081.01
16	–	9908049.06	000849.05	200108075.06	–	200108081.04
17	9908041.10	9908049.08	–	–	–	–
18	0008047.02	9908050.01	9908050.03	200108076.02	200108081.10	200108081.09
19	–	–	–	–	–	–
20	9508048.10	9508051.09	9300896.02	200100283.01	200334.09	–
21	–	–	–	–	–	–
22	–	9908051.04	–	–	200108082.08	200108082.06
23	0008040.03	–	–	–	–	–
24	–	–	–	–	–	–
25	–	–	9808024.09	200108076.08	–	200108083.01
26	–	0008050.05	0100471.07	–	200108083.04	200108083.05
NTN4-15[f]	–			200100336.01		
NTN6-14[f]	–			200100341.08		
NTN7-11[f]	–			200100342.12		
NTN10-19[f]	–			200100343.10		
NTN12-11[f]	–			200100423.08		
NTN16-15[f]	–			200100346.14		
NTN17-11[f]	–			200100357.03		

[a] Primary monosomic and monotelodisomic stocks from the cross between *G. hirsutum* (TM-1) and *G. barbadense* (3–79)

[b] Primary monosomic and monotelodisomic or tertiary monosomic stocks from the cross between *G. hirsutum* (TM-1) and *G. tomentosum*

[c] M: monosomic stock

[d] sh: monotelodisomic stock with short arm present

[e] Lo: monotelodisomic stock with long arm present

[f] NTN represent tertiary monosomic stocks of *G. hirsutum* (TM-1) crosses with *G. tomentosum*, they are missing a chromosome segment of two chromosomes involved in reciprocal translocation. For example, NTN 17-11 denotes that TM-1 chromosome lacks chromosome segments of chromosomes 17 and 11 in sub F_1 plant

at a greenhouse of USDA-ARS, Mississippi State, MS. Fresh leaves were collected from individual plant, frozen in liquid nitrogen, and then subjected to genomic DNAs extraction by Qiagen DNeasy plant maxi kit (Qiagen Inc, Valencia, CA, USA).

BAC-derived SSR marker chromosomal assignment

SSR primers were developed from the TM-1/*Hin*dIII BAC and TM-1/*Bam*HI BIBAC libraries (Yu et al. 2002a, b). Libraries were screened with four types of SSR oligo sequences ('CA', 'GA', 'TA', and 'GAA'). SSR-positive BAC clones were then sub-cloned and sequenced. Primer pairs were designed from the unique flanking sequences of the SSR loci and then used for chromosomal assignment. One hundred and ninety two unlabeled BAC-derived SSR primer pairs were synthesized initially for detection of polymorphism by 3.5% MetaPhor agarose gel electrophoresis between parents of cytogenetic stocks (TM-1, 3–79, and *G. tomentosum*). A total of 94 polymorphic BAC-derived SSR primer pairs between parents were then labeled with 6-FAM, HEX, NED, PET, or VIC at Applied Biosystems (Applied Biosystems, Foster City, CA, USA) for the genotyping on aneuploid cytogenetic stocks using an automated capillary electrophoresis system ABI3100 or ABI3730XL Genetic Analyzer with GeneMapper software 4.0 (Applied Biosystems, Foster City, CA, USA). PCR reactions were performed in 10μl volumes containing 10 ng of cotton template DNA, 1 × GeneAmp PCR Gold buffer (10 × , 150 mM Tris–HCl, pH 8.0, 500 mM KCl), 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.1μM of each single primer, 0.35 U of Taq polymerase (AmpliTaq, Applied Biosystems, Foster City, CA, USA). The PCR amplification profile consisted of an initial denaturation of DNA at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min. After 35 cycles, the extension temperature of 72°C was held for 8 min. The deletion analysis method was used for chromosomal assignment of BAC-derived SSR markers (Liu et al. 2000; Ulloa et al. 2005). The absence of the TM-1 (*G. hirsutum*) allele in any one of the hypo-aneuploid F_1 plant indicated the missing chromosome or chromosome arm was the most likely location of the marker (Fig. 1).

Results

Results showed that 175 out of total 192 primer pairs produced reliable amplified products on the three parental lines. The amplified SSR alleles ranged from 100 to 314 bp. A total of 76 polymorphic primer pairs were found between TM-1 and 3–79, whereas the number of polymorphic primer pairs was 59 between TM-1 and *G. tomentosum*. The chromosomal assignment results are summarized in Table 2. Overall, 39 BAC-derived SSR markers were located to 17 different chromosomes or chromosome arms. No markers could be assigned on the two homoeologous chromosome pair 9 and 23 by either series of the cytogenetic stocks.

Primary monosomic and monotelodisomic stocks deletion analysis

The chromosomal locations of the overlapped polymorphic markers between both parental combination (TM-1 and 3–79; TM-1 and *G. tomentosum*) were accomplished by both series of aneuploid stocks. However, there are also some of the SSR markers which are polymorphic only between TM-1 and 3–79 or between TM-1 and *G. tomentosum*. Under this situation, only one series of aneuoploid lines was used for chromosome location identification. Chromosome location of markers from primer pairs TMB0154, TMB1356, TMB1629, TMB1277, TMB1421, TMB0853, TMB1346, TMB2068, and TMB0564 were confirmed by both aneuploid stocks (Table 2). The conflicting chromosome location result had not been found by both series of aneuploid stocks. In addition, 16 more BAC-derived SSR markers were assigned to 12 different chromosomes by only aneuploid F_1 stock between TM-1 and 3–79. Similarly, 11 more markers were located to 8 different chromosomes using only aneuploid F_1 stocks between TM-1 and *G. tomentosum*.

Tertiary monosomic deletion analysis

The tertiary monosomic lines (NTN) provided information on the chromosome location in one of the two chromosomes involved in the translocation. However, the results from NTN analysis may not suggest exact location of the markers unless comparing

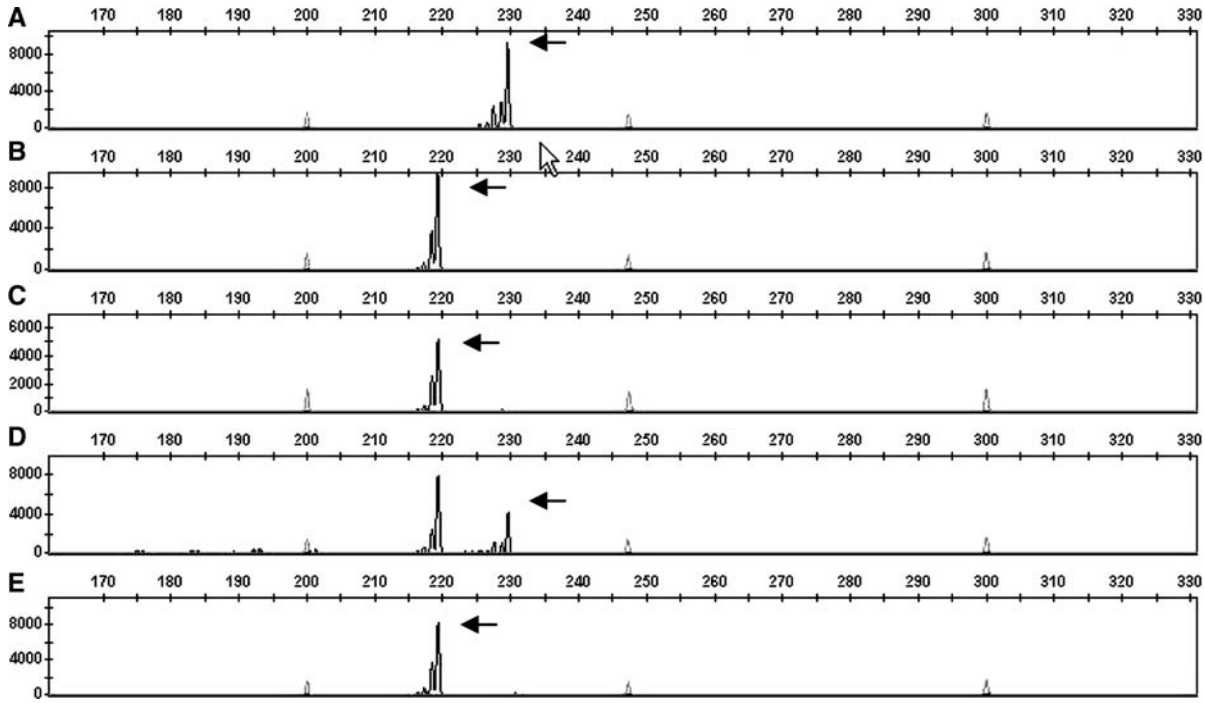

Fig. 1 Capillary electrophoresis results showing the chromosomal location of TMB1630 SSR markers. This marker was polymorphic between TM-1 (*G. hirsutum*) and 3–79 (*G. barbadense*) and allele size was 228 and 217 bp, respectively. (**A**) TM-1; (**B**) 3–79; (**C**) Aneuploid cytogenetic line with deficiency of chromosome 20 showing the missing of TM-1 allele; (**D**) Aneuploid cytogenetic line with deficiency of chromosome 20 short arm showing the presence of both parental alleles; (**E**) Aneuploid cytogenetic line with deficiency of chromosome 20 long arm showing TM-1 allele is missing

results from aneuploid stocks related to the chromosomes involved in NTN line. Primer pair TMB1348 showed missing TM-1 allele 148 on NTN6-14 and also Te14Lo on aneuploid *G. barbadense*, which suggested it was on short arm of chromosome 14. Similarly, the chromosome location of TMB1232 allele 189 was put on the short arm of chromosome 11. Primer pair TMB1664 missed TM-1 allele 188 in both NTN16-15 and NTN4-15. It indicated its possible chromosome location was 15. Molecular markers generated from primer pairs TMB2018, TMB0312, and TMB1295 could only find the deficiency in one tertiary monosomic stock. Thus, we could not find their exact chromosome locations, which could be on either of the two involved chromosomes (Table 2).

In conclusion, we discovered chromosome locations of 39 BAC-derived SSR markers in cotton. Among all the markers assigned to chromosomes, 19 markers were assigned to A-subgenomes (chromosome 1–13), 17 markers were assigned to D-subgenomes (chromosome 14–26). The subgenome status for the other three markers remained unclear because they could only be assigned to two potential chromosomes by tertiary monosomic stocks (Table 2).

Discussion

Cotton is the world's leading fiber crops. Two allotetraploid species, *G. hirsutum* and *G. barbadense*, are the most important cultivated cottons which united the A- and D-compound genomes together (AD; $2n = 4x = 52$) (Wendel and Albert 1992). The relatively large number of chromosomes and allotetraploid nature of cotton genome complicates cotton genetic mapping. Aneuploid stocks-based chromosomal assignment of markers and linkage groups was widely used in allopolyploid crops such as wheat (Werner-Fraczek and Close 1998) and cotton (Saha and Stelly 1994; Liu et al. 2000; Kohel et al. 2002; Rong et al. 2004). Recently, several SSR marker-based molecular maps have been

Table 2 Chromosome locations of BAC-derived SSR markers

Primer	Sequence (5′→ 3′)	Allele size (TM-1/3–79/*G. t*)[a]	Chromosome location[b]	
			3–79[c]	*G. t*[d]
TMB0062	F: GCATTGAAGGAAAAAGAAGAACC	245–248*/245–268/245	–	1
	R: ATGCCTTGTTTGCTTGAAGT			
TMB1421	F: TGCATATAATGCAAGAATTCCA	180*/167/186	1 sh	1 sh
	R: AGCAATTGGTATTAGAACTAGG			
TMB0605	F: AAATTAAACACGATTTCAAACGA	290*/295/290	3 Lo	
	R: TGTTTTGCATGCCTGGTACA			
TMB0564	F: ATTTCCATCACTTACACAC	101–196*/159/100–183	3 Lo	3 Lo
	R: CAGTAATCCTTAACTCAAG			
TMB0446	F: GCTTCTTTCTCTGGCTGCTG	176*-185/183/176–184	4 Lo	–
	R: GAAAGGGGGCTGATTTTGAG			
TMB0191	F: TGCGGTTTGAAATAGCATCAG	167–181–207*/167–175–189–214/ 167–185–196–202	5 sh	–
	R: GTTGCATCTTCGCTGTCTTG			
TMB1277	F: GCGAGAGGGAAGTTGTAATGTC	251*/263/263	6 Lo	6 Lo
	R: CCAACACACCAACACTCCAC			
TMB0154	F: TGTCAAGTTCAAGGGCACAA	258*/246/244	6 sh	6 sh
	R: TCCAAGTCCCACCATGAGTT			
TMB1346	F: ATGCTAAGTCTGACACATTGG	166–266*/274/169–274	6 Lo	6 Lo
	R: TCTCATTGCATCAACCGAAT			
TMB0853	F: CAAGTTCAAGGGCACAAAAT	249*/237/235	6 sh	6 sh
	R: CCCACCATGAGTTATTTCCA			
TMB1538	F: TTGTCAAGTTCAAGGGCACA	178–208*/196/194	6 sh	–
	R: TTAGTTCATAGTTTGGATTGATGC			
TMB0436	F: TGTGGCACAACCTTCCAAT	188–209*/169/169	–	6 sh
	R: CGTGTTCTCCATTTGATTCAT			
TMB0180	F: CAACCATCACACCCAACAAA	183*-196/175–194/183–194	7 sh	–
	R: AAAATGGAATGTTCCAGTCACC			
TMB1356	F: GTGTACATTGCGCTTTCGAG	199–238*/203/198	10 Lo	10
	R: TCCAAAATTTCAAGCCAACC			
TMB0380	F: CCCTACGCCCCTAATAGCAC	243*/236/232	10 Lo	–
	R: TCGAGTTACTTTTGGCAAGG			
TMB0325	F: GGAGCCTGGGTCTCTAGCTT	199*/186/176	10 Lo	–
	R: ACGGTGGTCTGGTGACTGA			
TMB0307	F: GGAGCCTGGGTCTCTAGCTT	144–198*/186/176	10 Lo	NTN10–19
	R: ACGGTGGTCTGGTGACTGA			
TMB0043	F: TTGCGTTTAGTTGATTTTCTAC	173–178*/175–182/163–175	–	11 sh
	R: CAATATCCCAGCCCTTTTCC			
TMB1660	F: GCATTGAATAATACTGGCTAAGAGC	118–127–198*/190/175	–	12 sh
	R: CAATAACAAATTTAGCCCATCG			
TMB1348	F: ACGATTGTGGAAAGAGATAGG	148*/141/141	14 sh	NTN6–14
	R: TCCGACCTGAAATCTGACCT			
TMB0201	F: GCTTGTTACGCTTCCACCA	202–226*/201/202–213	–	15 sh
	R: ATTGCTTTACGGCATCTGCT			

Table 2 continued

Primer	Sequence (5′→ 3′)	Allele size (TM-1/3–79/*G. t*)[a]	Chromosome location[b]	
			3–79[c]	*G. t*[d]
TMB1271	F: TCGATTAAAAATGAGCCTTGG	239*/192/210	16 Lo	–
	R: GGATACAATCTAATTTCATCCCAAT			
TMB2068	F: AAGTTTTCGGCTCCCTCACT	150*/133/147	16 sh	16 sh
	R: GCTGCTGGGGACTATTCTTG			
TMB0564	F: ATTTCCATCACTTACACAC	101*-196/159/100–183	16 sh	–
	R: CAGTAATCCTTAACTCAAG			
TMB0874	F: AAATAGAAACACAGAAAAAAAAATA	198*/187/198	17 Lo	–
	R: AGACCAGCTGTGTTCTAGTA			
TMB0029	F: TAGGCATAACCAACATGACC	197–203–212*/197–212/197–202	–	18 Lo
	R: TGGCTAGGTGGTATAAACTGAG			
TMB1638	F: AAAACCAAGAATCGAGGAAAAA	152*-183/152–156/153–162	–	18 sh
	R: TGCAATCCTCGAAGGTCTTT			
TMB1664	F: AAATACCGGAACTTGATTGGG	188–205*/193/156	18 sh	–
	R: AATTTGGTTGGGTTTCCACA			
TMB1630	F: TCCATGGAAATCCATCAACA	228*/217/224	20 Lo	–
	R: ACCCAAGTTGCAGCTGTTTC			
TMB1313	F: TCCCTTTTGTTTCCTTGTGG	188–194*/183–188/188–193	20 sh	–
	R: TCCTATTCAATTCAGGGCTTC			
TMB1629	F: TTCCAAGGTTTGCCTTTGTT	239*/314/232	20 sh	20 sh
	R: TCATGAAAGAGATAAAGGAGAAAAGA			
TMB0443	F: AAGTTGCAGGTCTTTCTC	257*/247–259/247–271	–	20 Lo
	R: ACCATCCATACCATCATC			
TMB0313	F: CCTGTTTATGCTGCCTTTGA	164–199*/164–173/164–173	–	25
	R: CAATACCCATGCTTGGTTCC			
TMB0366	F: GAGCCCACCATTATCACTCC	201–206*-216/201–216/199–212	26 Lo	–
	R: GGTGGTCATGTGAGAGAGGA			
TMB1664	F: AAATACCGGAACTTGATTGGG	188*-205/193/156	–	NTN16–15; NTN4–15
	R: AATTTGGTTGGGTTTCCACA			
TMB1232	F: TTACCAACTCCAAATCTGTAAC	189*-201/256–281/204	–	11sh; NTN12–11
	R: CGATCAGAATCCAAGCACAG			
TMB2018	F: GCTCCATTGGTTGCAGGTAT	228–231*/228–256/228–237	–	NTN17–11
	R: CATGAAGTTGAAAGAAGCAGCTA			
TMB0312	F: AGCTTTTCCATTCCAGAGCA	173–206*/173–193/173–193	–	NTN12–11
	R: GGTTGTTGCAAGAGTTCACG			
TMB1295	F: CTGATCCAAACACCCATGC	217–223*/217–221/211–218	–	NTN10–19
	R: CGTGGAATTTGGTCATTTTG			

[a] Allele size presents as the order TM-1/3–79/*G.t*; *G. t* indicate *G. tomentosum*; allele marked by "*" had been assigned chromosomal location

[b] Chromosome location with Lo and sh represent long arm and short arm, respectively

[c] Chromosome location identified by aneuploid F_1 hybrids between TM-1 (*G. hirsutum*) and 3–79 (*G. barbadense*)

[d] Chromosome location identified by aneuploid F_1 hybrids between TM-1 (*G. hirsutum*) and *G. tomentosum*

published in cotton (Han et al. 2004, 2006; Nguyen et al. 2004; Lacape et al. 2005; Park et al. 2005; Song et al. 2005; Frelichowski et al. 2006; Guo et al. 2007). Even though the most saturated SSR marker-based linkage map reached an average 1.91 cM inter-marker distance (Guo et al. 2007), most of these markers were from expressed sequence tags (EST) and reflected gene-rich regions, which may not cover the whole genome of cotton. Lacape et al. (2003) proposed that recombination hot spots along the chromosome, biased distribution of available markers, and insufficient marker number would lead to uncompleted coverage of whole genome. Developing new SSR markers from a BAC library has the potential to populate currently relatively blank regions of the genome, which may be targeted by conventional marker development methods (Liu et al. 2000; Lichtenzveig et al. 2005; Frelichowski et al. 2006). Additional BAC-derived SSR markers will help to saturate current genetic linkage maps of cotton. The 39 BAC-derived SSR markers loci which localized to specific chromosomes and/or chromosome arms in this experiment could be ideal candidates as framework markers to extend preexisting linkage maps.

Considering the correspondence between BAC-derived SSR markers and their original clones, chromosomal assignment of these markers will facilitate the integration of physical maps with genetic maps and the coordination of chromosome-based genome sequencing (Danesh et al. 1998; Cregan et al. 1999; Chen et al. 2002; Yu et al. 2004b, Wu et al. 2004). Yu et al. (2002a, b) reported the development of these set of BAC-derived SSR markers and their importance to bridge physical contig maps with genetic linkage maps. Their following studies (Yu et al. 2004a, b) showed the ongoing research toward a whole-genome physical map of cultivated allotetraploid cotton by an automated procedure. Wang et al. (2006, 2007) provided a classic example how to integrate genetic and physical maps in tetraploid cotton using SSR markers by fluorescence in situ hybridization (FISH). However, majority of these markers were genomic SSRs developed by conventional methods and EST-SSRs. The direct relations among BAC-derived SSR markers, their chromosomal locations, and original clones will increase the efficiency and effectiveness in the integration of linkage and physical maps in tetraploid cotton (Yu et al. 2004a, b). Although some TM series BAC-derived SSR markers were associated to chromosomes by linkage mapping in previous reports (Han et al. 2004, 2006; Song et al. 2005; Guo et al. 2007), we used capillary electrophoresis system to screen microsatellite and standard nomenclature documented in CMD. Abdurakhmonov et al. (2007) used some of these BAC derived SSR markers to associate with photoperiod related mutation. Our study provided valuable information on the chromosomal locations of these markers. The concordance comparison between linkage mapping and deletion analysis could not be performed in this study.

In this experiment, most of the primer pairs produce one polymorphic locus among three cytogenetic stock parents, which could be chromosomally assigned. However, two polymorphic SSR loci had been amplified from each primer pair of TMB0564 and TMB1664. The 196 bp locus of TMB0564 was located to the long arm of chromosome 3 by both series of aneuploid stocks (TM-1 and 3–79; TM-1 and *G. tomentosum*), whereas the 101 bp locus of TMB0564 was assigned to the short arm of chromosome 16. As to primer pair TMB1664, the 188 bp locus was localized to chromosome 15 by two tertiary monosomic stocks, but the other 205 bp locus was put on short arm of chromosome 18. However, no homeologous relationship could be found between chromosome 3 and 16 or between chromosome 15 and 18 (Wang et al. 2006). Rong et al. (2004) observed several duplication events within each subgenome in addition to homoeologus duplication in cotton. They suggested that this could be due to retrotransposition or present-day cotton may be derived from a putative ancestor containing six or seven chromosomes. In another linkage mapping study, Han et al. (2006) located marker BNL3590_180 on chromosome 2, but the other two loci generated from the same primer pair BNL 3590_215 and BNL3590_265 were on chromosome 17. Same situations happened on loci amplified using primers CIR094 and NAU0667 (Han et al. 2006). Previously studies on cotton functional genes analyses (Pfeil et al. 2004; An et al. 2007) also suggested that there were more events of duplication in cotton genome in addition to the polyploidy event in the evolution of tetraploid cotton species.

Parental screening results revealed 76 and 59 polymorphic primer pairs between TM-1 and 3–79 or

G. tomentosum, respectively. However, only 39 BAC-derived SSR markers had been located on chromosomes by either series of the aneuploid cytogenetic stocks. The remaining polymorphic markers could not be assigned to specific chromosomes possibly due to the following reasons: (1) lack of complete chromosome coverage of substitution stocks; (2) the slightly difference among individual vegetative copies of substitution line which may modified by some epigenetic factors; (3) some residual effect of the original aneuploid when it was backcrossed with TM-1 to recover the hypoaneuploid TM-1 isogenic parent for the specific chromosome; or (4) chromatin losses during backcrossing or other events of unknown cytological abnormalities in the development of these cytogenetic stocks.

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